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Dynamics of *Nosema pyrausta* in natural populations of the European corn borer, *Ostrinia nubilalis*: A six-year study

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Abstract. *Nosema pyrausta* (Paillot) (Microsporida: Nosematidae) is an obligatory intracellular parasite of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae). This pathogen is maintained in natural populations of *O. nubilalis* by both horizontal and vertical transmission. The impact of *N. pyrausta* on fecundity of adults and survival of larvae has been well documented in laboratory and field research. In an extensive study covering a 6 year period at one site, we described the effect of *N. pyrausta* within *O. nubilalis* populations in a continuous corn following corn ecosystem. We documented the presence of the pathogen through all life stages of *O. nubilalis* (egg, larva, pupa, adult), by collecting throughout the crop season and examining each insect stage in the laboratory for the frequency of infection with *N. pyrausta*. The percentage of infection with *N. pyrausta* and magnitude of the *O. nubilalis* population fluctuated throughout generation 1 and generation 2. Both horizontal and vertical transmission played a role in maintaining *N. pyrausta* in the population in both generations. There were strong correlations between percentage adults with *N. pyrausta* and percentage larvae with *N. pyrausta*, and between percentage eggs with *N. pyrausta* and percentage larvae with *N. pyrausta*. There was a weak correlation between percentage adults with *N. pyrausta* and percentage eggs with *N. pyrausta*. The percentage of insects infected with *N. pyrausta* was always lowest in the egg.

Key words: Crambidae, horizontal/vertical transmission, Lepidoptera, Microsporida, Nosematidae, *Nosema pyrausta*, *Ostrinia nubilalis*

Introduction

Nosema pyrausta (Paillot) (Microsporida: Nosematidae) is an obligatory intracellular parasite of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae). Because of its impact on

fecundity of adults and survival of larvae it is one of the primary natural regulators of *Ostrinia nubilalis* populations (Zimmack et al., 1954; Zimmack and Brindley, 1957; Kramer, 1959; Van Denburgh and Burbutis, 1962; Windels et al., 1976; Siegel et al., 1986; Solter et al., 1990; Sajap and Lewis; 1992). *Nosema pyrausta* is maintained in natural populations of *O. nubilalis* by both horizontal and vertical transmission (Zimmack et al., 1954; Zimmack and Brindley, 1957; Kramer, 1959; Lewis, 1978; Hill and Gary, 1979; Andreadis, 1984, 1986, 1987; Lewis and Cossentine, 1986).

Limited work has been published on the field ecology of *N. pyrausta* i.e., what biological or cultural mechanisms influence presence of *N. pyrausta* during and between generations of *O. nubilalis*. In an intensive study covering a 6 year period at one site, we describe the ecology of *N. pyrausta* within *O. nubilalis* populations in a corn following corn agroecosystem by documenting the existence of the microsporidium through all life stages of *O. nubilalis*.

Materials and methods

During a 6 year period (1985–1990), *O. nubilalis* were collected from a corn ecosystem on the R. LeRoy Sparks farm located 4.8 km southeast of Baxter, IA. Corn was planted in rows on 1.0 m centers in a corn following corn regime using accepted agronomic practices of herbicides and fertilizers during the extent of the research.

Each year of the study, all *O. nubilalis* life stages were collected throughout the growing season (Table 1). The methodologies used to collect each insect-life stage and document presence or absence of *N. pyrausta* were: *Adults* – During the first and second generations insects were collected using a black light trap located near the corn field edge. Adults were removed from the light trap 3 times per week during each generation. All adults in each collection were sexed and counted. Up to 25 males and 25 females of each collection were randomly selected. The percentage of mated females was determined by the presence or absence of a sclerotized spermatophore in the bursa copulatrix (Showers et al., 1974). The percentage of both sexes infected with *N. pyrausta* was determined by removing the Malpighian tubules and examining them with the aid of a 400× phase contrast microscope for the presence or absence of *N. pyrausta* spores. *Egg masses* – Ten consecutive corn plants were examined from 10 random sites in the field for presence of *O. nubilalis* egg masses. All egg masses found were collected, placed in 30 ml plastic cups, and returned to the laboratory.

Table 1. Numbers of European corn borer life stages collected in the field and examined for infection with *Nosema pyrausta*

Generation	Year	Larvae	Egg masses	Individual eggs[a]	Adults
First	1985	118	70	343	211/186[b]
Second		63	33	63	699/196[b]
First	1986	128	3	44	237
Second		125	112	1557	250
First	1987	131	1	16	98
Second		501	51	588	1675/614[b]
First	1988	18/12[b]	8	53	85
Second		410	23	343	89
First	1989	698/694[b]	152	1960	76
Second		1002	55	387	569/175[b]
First	1990	160	46	396	641/396[b]
Second		881	155	1272	4193/479[b]

[a]Number of eggs examined for infection with *Nosema pyrausta*.
[b]When number collected differed from number examined, the number collected is followed by the number examined.

Egg masses were collected 2–5 times each generation depending on the length of the oviposition period. In 1985, presence of *N. pyrausta* in eggs was determined by incubating the egg mass at 27 °C in the laboratory until hatch. At this time wet mounts were made of neonatal larvae which were examined for the presence or absence of *N. pyrausta* spores. An infected larva was interpreted as an infected egg. From 1986 to 1990 masses were separated into individual eggs by incubating in a 2% trypsin solution in a buffer of 0.05 M K_2 HPO_4 and 0.05 M KH_2PO_4 (pH 8) (Sajap and Lewis, 1992). Wet mounts of individual eggs were examined for presence or absence of *N. pyrausta*. *Larvae* – Once egg hatch had occurred in the field, larvae were collected during both generations by examining 10 plants at each of 10 randomly selected sites in the field for infestation by *O. nubilalis*. All infested plants were dissected; the larvae were collected, placed individually in 30 ml plastic cups, placed in a cooler and returned to the laboratory. Individual insects were classified to instar and the number of *N. pyrausta* spores per mg of infected tissue determined (Raun et al., 1960).

Statistical analyses

Analysis of variance (ANOVA) was used to examine the effects of year, generation, life stage and their interaction on the percentage

infection of individuals. Descriptions for each model tested are described below. Proc Mixed (Littell et al., 1996) of SAS, version 8.2 (SAS Institute Inc., 2003), was used to conduct ANOVA via restricted-maximum likelihood methods. After an examination of residuals versus predicted values, it was determined that a data transformation was necessary to meet the assumptions for ANOVA. All percentage data were arcsine-square root transformed for analysis.

To determine if the infection of individuals was dependent on year, generation, and life stage (adult, egg, early larval development, 1st–3rd instars, late larval development, 4th and 5th instars), the mean percentage infections for each life stage during each year and generation were calculated. The mean percentages were subjected to ANOVA with year, generation, life stage and their two-way interactions considered fixed effects. To examine vertical transmission patterns of infection, orthogonal, linear contrasts were used to partition effects associated with life stage and interaction effects including life stage. The following life stage comparisons were made: (1). percentage infection in adults versus eggs; (2). percentage infection in early larval versus late larval development and (3). remaining life stage effects.

The dynamics of percentage infection across years and generations were also investigated separately for each life stage. Year and generation were considered fixed effects and week nested within a year and generation was considered as a random effect. In addition to the above independent variables, sex of moths was included in the analysis of percentage infection in adults. Significant interaction effects were further examined via the "slice" option of the LSMEANS statement of Proc Mixed. The simple main effect of generation was examined for each year separately. Partitioning of interaction terms via orthogonal contrasts were used in some instances to examine the relative importance of the causes of significant F values.

The analyses above indicated that the infection of larvae by *N. pyrausta* was increasing during both generation 1 and generation 2. Path analysis (Wright, 1968) was used to better describe the direct and indirect causes of increasing infection during the larval stage. This method partitions correlations into direct and indirect estimates of causal relationships. Figure 1 defines our path model and the variables included were: percentage infection at Julian date x_t, percentage infection at the previous sample date ($x_{t\text{-}1}$), larval density at x_t, and Julian date. Arrows with single heads indicate a direct effect of the variable at the base of the arrow on the variable at the point of the arrow. Double-headed arrows indicate the variables may covary due

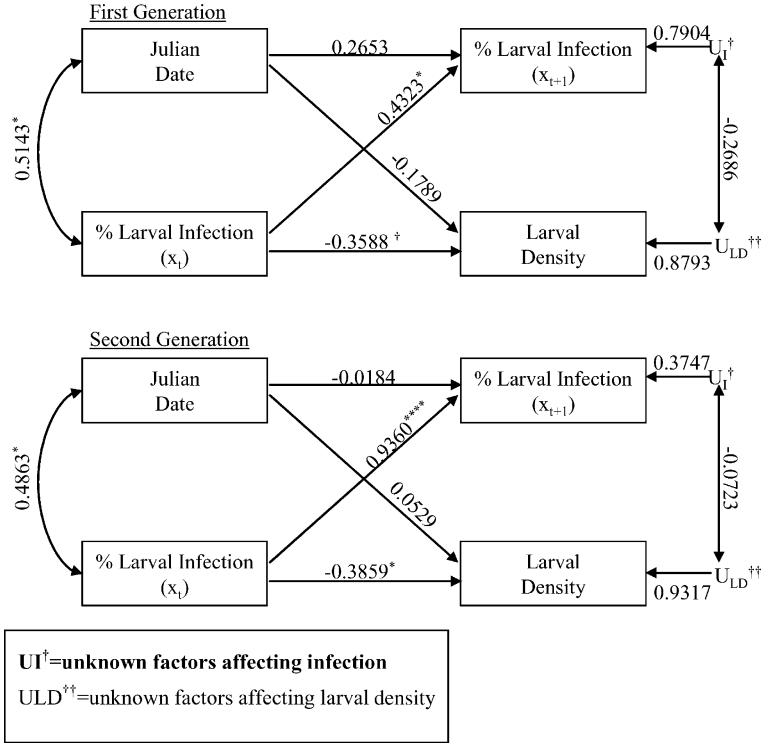

Figure 1. Path model for larval density and percentage infection of larvae during generation 1 and generation 2. Variables represented with the letter "U" denote factors of unknown or unmeasured effect on the variable at the end of the arrow. $^{\dagger}p < 0.10$, $^{*}p < 0.05$, $^{****}p < 0.0001$.

to unknown background effects. Julian date is considered as a variable that accounts for seasonal abiotic and biotic factors that cannot be accounted for by the data that were collected. Causal links involving larval infection at x_{t-1} are a measure of the build up of infection and its transmission within a generation. Proc Calis (SAS Institute, 2003) was used to estimate path coefficients as well as test the goodness-of-fit of the path model. In addition, multivariate regression via Proc Reg (SAS Institute, 2003) was used to test the significance of the path coefficients. Estimates of path coefficients were the same in Proc Calis and Proc Reg.

Results

ANOVA revealed significant differences in the mean percentage of insects infected with *N. pyrausta* among years, life stages, and year ×

generation interactions (Table 2). Differences among life stages were due to a decline in the percentage infection of eggs relative to adults and an increase in the infection of larvae during a generation (Figure 2). None of the differences among life stages in the percentage of insects infected with *N. pyrausta* were dependent on year × life stage and generation × life stage (Table 2).

The percentage infection of adults was evaluated for the effects of year, generation, sex and their interactions (Table 3). The infection of adults was significantly affected by year (Table 3). During 1989 fewer adults were infected by *N. pyrausta* (Figure 3). No overall differences in adult infection were found between the first and second generations. However, differences between generations were dependent on year. During 1986 and 1990, there was greater infection in adults during the second generation than the first generation (Figure 3, Table 3). During all other years first-cycle adults exhibited greater levels of infection. Slicing of Year × Generation least-squared means by

Table 2. ANOVA testing for the effects of year, generation, and life stage of *Ostrinia nubilalis* on the percentage infection by *Nosema pyrausta*. Percentages were arc-sin – square root transformed for the analysis. Effects in model were further partitioned into contrasts, see Materials and methods

Source of variance	NDF[a]	DDF[b]	F	*p*
Year	5	15	4.26	0.0130
Generation	1	15	2.18	0.1606
Life stage	3	15	14.89	< 0.0001
Adult versus Egg	1	15	21.83	0.0003
Larval stage: Initial versus Final	1	15	21.91	0.0003
Remaining life stage effects	1	15	0.94	0.3470
Year × Generation	5	15	3.93	0.0178
Year × Life stage	15	15	1.33	0.2954
Year × (Adult versus Egg)	5	15	0.86	0.5327
Year × (Larval stage: Initial versus Final[c])	5	15	1.97	0.1420
Year × (Remaining life stage effects)	5	15	1.16	0.3750
Generation × Life stage	3	15	0.70	0.5673
Generation × (Adult versus Egg)	1	15	1.32	0.2680
Generation × (Larval Stage: Initial versus Final)	1	15	0.42	0.5257
Generation × (Remaining life stage effects)	1	15	0.35	0.5628

[a]Numerator degrees of freedom.
[b]Denominator degrees of freedom.
[c]Larval stage: Initial = 1st–3rd, Final = 4th–5th.

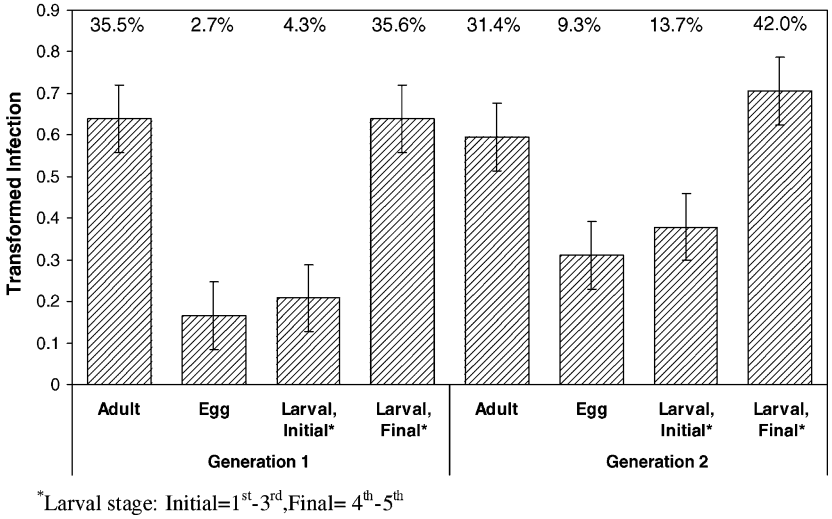

Figure 2. Least-squared means (±SE) of the arcsine-square root transformed percentage infection by *Nosema pyrausta* for life stages during the first and second generations. Values above each bar represent the back-transformed percentage infections.

Table 3. ANOVA testing for the effects of year, generation, and sex of on the percentage infection of *Ostrinia nubilalis* adults by *Nosema pyrausta*. Percentages were arc-sin – square root transformed for the analysis. Effects in model were further partitioned into contrasts, see Materials and methods

Source of variation	NDF[a]	DDF[b]	*F*	*p*
Year	5	28	11.86	< 0.0001
1989 Versus other years	1	28	52.57	< 0.0001
Remaining year effects	4	28	1.30	0.2931
Generation	1	28	0.17	0.6843
Sex	1	28	< 0.01	0.9465
Year × Generation	5	28	4.46	0.0041
(1986 & 1990 Versus Other years) × Generation	1	28	19.59	0.0001
Remaining year × Generation effects	4	28	0.71	0.5915
Year × Sex	5	28	1.82	0.1415
Generation × Sex	1	28	0.02	0.8878
Year × Generation × Sex	5	28	0.48	0.7845

[a]Numerator degrees of freedom.
[b]Denominator degrees of freedom.

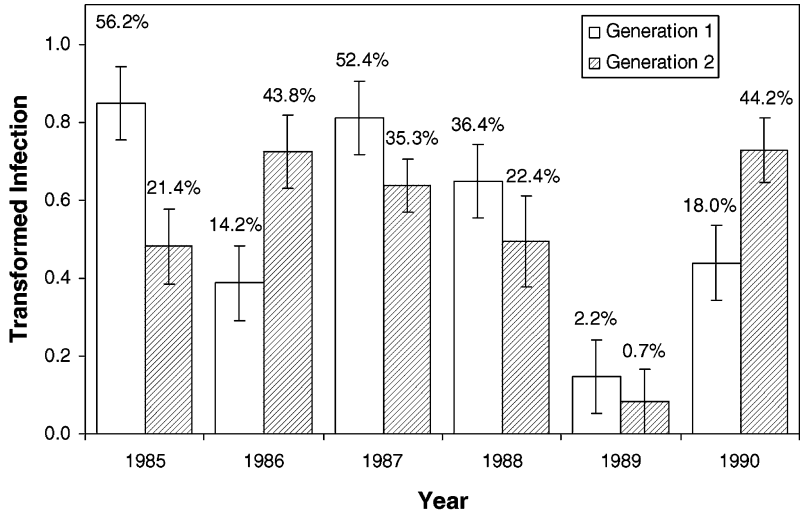

Figure 3. Least-squared means (±SE) of the arcsine-square root transformed percentage infection of adults by *Nosema pyrausta* for years during the first and second generations. Values above each bar represent the back-transformed percentage infections.

year revealed significant differences between generations during 1985 ($p = 0.0111$), 1986 ($p = 0.0184$), and 1990 ($p = 0.0296$). There were no differences in the percentage infection of males and females.

The percentage of eggs infected by *N. pyrausta* significantly varied among years ($F = 11.57$; df = 5, 25; $p < 0.0001$). Differences among years were due to greater percentages of eggs being infected during 1985 ("1985 versus other years" $F = 47.44$; df = 1, 25; $p < 0.0001$). All other years exhibited similar percentage infections of eggs ($F = 2.05$; df = 4, 25; $p = 0.1176$). No significant differences were present between generations 1 and 2 ($F = 3.38$; df = 1, 25; $p = 0.0778$) nor was there an interaction between generation × year ($F = 1.63$; df = 5, 25; $p = 0.1887$).

Larval infection was dependent on year ($F = 13.77$; df = 5, 45; $p < 0.0001$), generation ($F = 23.97$; df = 1, 45; $p < 0.0001$) and the interaction between year × generation ($F = 7.35$; df = 5, 45; $p < 0.0001$). During all years, there was a greater percentage infection of larvae during the second generation than during generation 1 (Figure 4). The difference between generations was especially great during 1987 and 1990 ("[1987, 1990 versus other year] × generation" $F = 20.40$, df = 1, 45; $p < 0.0001$; "[Differences among 1985, 1986, 1988, 1989] × generation" $F = 0.51$; df = 3, 45; $p = 0.6762$). The degree of the difference between generations also differed between 1987 and 1990 ("[1987

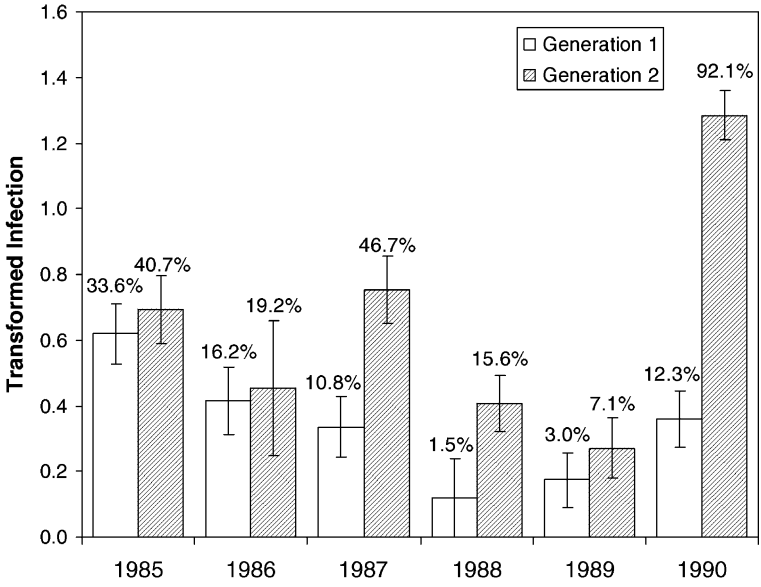

Figure 4. Least-squared means (±SE) of the arcsine-square root transformed percentage infection of larvae by *Nosema pyrausta* for years during the first and second generations. Values above each bar represent the back-transformed percentage infections.

versus 1990] × generation" $F = 8.18$; df = 1, 45; $p = 0.0064$). Physical slicing of the year × generation effect revealed generations were significantly different only during 1987 and 1990.

To better examine horizontal transmission of *N. pyrausta* infection during the larval stage, path models were designed to delineate factors potentially affecting infection (Figure 1). The models reported provided adequate fit to the data ($p > 0.9$ for both generations). The Julian date is a measure of variables that cannot be accounted for by the data collected (e.g., seasonal abiotic and biotic factors). The percentage of larvae infected by *N. pyrausta* always increased within a generation. During generation 1, the only significant influence on the percentage infection of larvae was the percentage infection of the previous sampling date (Figure 1). There was no significant direct effect of Julian date on the percentage of larvae infected during generation 1. Larval density was marginally affected in a negative direction by the percentage infection of larvae at the previous sample date.

During generation 2, the percentage infection of larvae was strongly affected by the percentage of larvae infected at the previous sample

date. Larval density was negatively impacted by the direct effect of the percentage infection of larvae at the previous sample date. There was a significant, negative relationship between the direct effects of larval density and the percentage infection of larvae during generation 2 ($r = -0.3622$).

Discussion

This research is unique compared with other reports of in-field studies because we recorded *N. pyrausta* infection from all stages of the insect for 6 successive years. In other studies larval stages, adults and larvae, or only adults were evaluated (Hill and Gary, 1979; Andreadis, 1986; Seigel et al., 1988). These noted studies, however, are instrumental in our understanding of the maintenance of *N. pyrausta* in a wild population. The mechanism of maintenance of spores is vertical (transovarial) and horizontal (larva–larva) transmission. A brief overview of this biology is that an *O. nubilalis* adult infected with *N. pyrausta* oviposits eggs containing spores. As the eggs embryonate the spores infect susceptible tissues, i.e., primarily midgut, Malpighian tubules, and fat body. At eclosion a high percentage of the larvae are infected with *N. pyrausta*. As they develop through subsequent instars, the number of spores continues to increase, infecting additional tissues and eventually infecting reproductive tissues, prerequisite for passing of spores to the filial generation. As *N. pyrausta* infected larvae feed, midgut cells rupture and cellular content including spores are sloughed into the midgut and are passed in fecal material becoming a source of inoculum to infect larvae feeding in the vicinity. The success of the above processes is dependent on many biotic and abiotic occurrences. The magnitude of these occurrences drives the fluctuations of *N. pyrausta* in populations of *O. nubilalis*.

Because our data include all life stages they provide the opportunity to define the role of each of these stages in maintaining *N. pyrausta* in a wild population of *O. nubilalis*. In IA, the generation 1 *O. nubilalis* adult is a product of generation 2 of the previous year, as it passes the winter as a diapausing 5th instar, and develops to a pupa and adult in the spring. We will begin with this adult and follow the stages of development through generation 1 to the diapausing larva of generation 2.

Percentage of infection in adults varied between generations within a year and between generations between years (Figure 3, Table 3). In all years except 1986 and 1990, the percentage infection with

N. pyrausta was greater in adults of generation 1 than in adults of generation 2. Over years, the percentage of infection in generation 1 adults ranged from 62.9 (1985) to 16.0 (1989) and was 36.5 averaged over years. Correspondingly, in generation 2, the percentage infection in adults ranged from 53.6 (1990) to 4.5 (1989), and was 33.1 averaged over years. The number of adults infected with *N. pyrausta* collected from generation one is not significantly correlated with the number of infected adults collected from generation two. There is also no correlation between the number of second generation larvae infected with *N. pyrausta* and adults in generation one the next year. Theoretical models indicate that regional adult movement can have impact on the dynamics of *N. pyrausta* infection (Onstad and Maddox, 1989; Onstad et al., 1990). There is little evidence that percentage infection in adults changes once the insects are in this stage. Evidence of venereal transmission is inconclusive; however, there is evidence from laboratory experiments that adults can become infected by consuming spores (Solter et al., 1991). These authors speculate that the likelihood of this happening in nature is limited. The intensity of infection in an adult female is dependent on the larval stadium in which an infection occurs, and on the dosage of *N. pyrausta* spores (Sajap and Lewis, 1988, 1992).

Ostensibly, the frequency and intensity of infection in the female determines the same in the egg. The percentage *N. pyrausta* infection in eggs was equally variable. It was greater in eggs in generation 2 than in generation 1, except in years 1985 and 1986 ranging from 21.0 (1985) to 0.0, the average over years of 5.5 (1987, 1988) and 37.4 (1985) to 3.1 (1989) average of 11.1 over years, respectively, for generations 1 and 2. Substantial laboratory research has been conducted on vertical (adult to egg) transmission. *N. pyrausta* infection in the adult contaminates the oocytes resulting in the laying of infected eggs (Kramer, 1959). The percentage of infection in the adults unequivocally influences the percentage of infection in the eggs (Sajap and Lewis, 1988). Also, frequency of infection in the eggs is dependent on the time within the oviposition period an egg is laid; eggs laid on day 3 of the oviposition period produce significantly more infected neonates than eggs laid on day 1 (Siegel et al., 1986; Sajap and Lewis, 1992). A possible explanation for the consistent decline of percentage infection from adult to egg is that the more heavily infected females lay fewer eggs and the eggs laid early in the oviposition period have a lower percentage infection. And it is known that a female *O. nubilalis* lays the majority of its eggs early in the oviposition period (Sajap and Lewis, 1992). Another possibility is that cool weather influenced the

percentage infection. In laboratory studies in which temperature $\times N$. *pyrausta* infections were investigated it was determined that some heavily infected females died without laying eggs and others laid significantly fewer eggs, making the probability of collecting an infected egg much less than collecting a non infected one (Bruck et al., 2001).

Percentage of larvae infected with *N. pyrausta* differed between years and generations. The infection ranged from 35.6 (1986) to 4.0 (1989) in generation 1 (average = 16.6) and 85.8 (1990) to 7.8 (1989) in generation 2, (average 34.8). Infections in larvae are dependent on the percentage of eggs infected and then horizontal transmission within larvae or within the plant. Biology of the *O. nubilalis* larvae in the first generation is that once the egg ecloses the larva moves into the leaf whorl of vegetative corn feeding at the interphase of the unfurling leaves and in the late 4th to early 5th instar bores into the plant. When eggs are laid on a reproductive-stage plant (2nd generation) the neonatal larva commence feeding at the junction of the leaf axil and sheath collar and then behind the leaf-sheath collar prior to boring into the stalk (Mason et al., 1996). Once an infected larva begins feeding it deposits *Nosema* spores in its fecal material (Andreadis, 1986; Solter et al., 1990), which allows for horizontal transmission. Thus, the percentage of *N. pyrausta*-infected larvae increases in later instars in both the first and second generation. Other studies also show high variability in frequency of infection in larvae over years within locations and over geographical locations. Lewis (unpublished data) observed infections ranging from 0 to 100% within locations over a 3 year period. They also reported similar variations across locations and production practices.

The eggs examined in this study always had the lowest average percentage infection of *N. pyrausta* with percentage infection in the subsequent larvae always increasing. The model, Figure 1, illustrates a significant correlation between percentage infection in early instars and percentage in late instars in both generation 1 and 2, documenting horizontal transmission. Strong horizontal transmission of *N. pyrausta* in *O. nubilalis* in generation 1 has not been reported by others; however, Seigel et al. (1988) documented an increase in larval infection in generation 2 as the result of horizontal transmission. Andreadis (1986) concluded that low population densities limited horizontal transmission in generation 1. Likewise, in this study the correlation indicative for horizontal transmission was much greater in generation 2 than generation 1.

Several biotic factors can influence increase in percentage of population infected with *N. pyrausta* throughout a growing season.

Diapausing larvae infected with *N. pyrausta* have a higher percentage of mortality during the winter than those not infected (Lewis, unpublished data). The initial inoculum and percentage infection in the overwintering insect determines the frequency of *N. pyrausta* in generation 1 insects. Any heavily infected females under cool spring weather conditions have died before ovipositing, or the infected ones laid very few eggs compared to the non infected ones (Bruck et al., 2001). As the larvae develop the indigenous spores go through generations increasing the intensity of the infection, i.e., spores/g of insect. The generation time of spores is dependent on temperature and inoculum (Maddox, 1968). Infected larvae deposit *N. pyrausta* spores in their feces soon after they begin feeding and as intensity increases voided fecal matter will carry a greater number of spores (Solter et al., 1990). Infection will then be transmitted to non infected larvae feeding in the same area. Also, if infected larvae consume contaminated frass they will increase their intensity of infection (Lewis et al., 1983). In generation 2 the previous occurrences are in force plus the percentage of infection can be increased when non infected larvae come in contact with *N. pyrausta*-contaminated frass remaining from the previous generation (Lewis and Cossentine, 1986). Larvae also have a great propensity to migrate to adjacent plants and either disseminate *N. pyrausta* or obtain an infection (Lewis, 1978). As the above scenarios are playing out the opportunity for an increase in percentage of a population having an infection increases. In addition, the larval period in generation 2 is substantially longer than that of larvae in generation 1 allowing for increased horizontal transmission (Andreadis, 1987; Siegel et al., 1988).

Larval density also influences horizontal transmission. Ostensibly greater the density greater the transmission. Hill and Gary (1979) concluded that high host density and widespread-spatial distribution of spores facilitate increased percentage of population becoming infected with *N. pyrausta*. Andreadis (1986) also concluded that larval density was a primary contributor to horizontal transmission.

Nosema pyrausta is a significant biological control agent in its own right. Another attribute is its success in concert with indigenous macro biological control organisms. *Macrocentrus cingulum* (Hymenoptera: Braconidae) is the most prevalent of introduced parasitoids (Lewis, 1982) parasitizing up to 20% of *O. nubilalis* in a given year and location. When *M. cingulum* parasitizes a *N. pyrausta*-infected *O. nubilalis* larva the results vary from no negative effect (Cossentine and Lewis, 1987) to causing a debilitation of the parasitoid (Andreadis, 1986). Also *Chrysopa carnea* and *Chrysopa oculata* develop to quality adults

when their sole food source is *N. pyrausta*-infected *O. nubilalis* eggs. In addition the *N. pyrausta* spores remain viable after passing through the Chrysopidae larvae, a novel way of disseminating *N. pyrausta* (Obrycki et al., 1989).

Nosema pyrausta has been documented as an ideal biocontrol agent i.e., maintains itself in wild populations of *O. nubilalis* while causing reduction in fecundity and larval survival. Even so there have been limited attempts to use *N. pyrausta* as a crop protection type of insect control (Lewis and Lynch, 1978; Lublinkhof et al., 1979; Lewis et al., 1982; Lewis, 1982). In all of these projects *N. pyrausta* was successful in reducing damage caused by *O. nubilalis*. Even with these examples of success the underlying point is that *N. pyrausta* is an obligatory parasite therefore propagation is *in vivo* only. No success in *in vitro* reproduction has been accomplished to support commercialization of *N. pyrausta*. The conventional wisdom is to learn as much as possible about the dynamics of *N. pyrausta* and develop the knowledge base to conserve the organism within wild populations of *O. nubilalis*.

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